Reference: *Biol. Bull.* **174:** 163–171. (April, 1988)

Control of Cnida Discharge: I. Evidence for Two Classes of Chemoreceptor

GLYNE U. THORINGTON AND DAVID A. HESSINGER[1]

Department of Physiology and Pharmacology, School of Medicine, Loma Linda University, Loma Linda, California 92350

Abstract. Appropriate chemical stimulation of cnidocytes along with mechanical stimulation is required to trigger discharge of cnidae. It has been generally assumed that such chemosensitization is mediated via specific chemoreceptors. Such chemoreceptors and their complementary ligands have never been identified. We now identify two groups of naturally occurring substances that chemosensitize cnida discharge in the feeding tentacles of the sea anemone, *Aiptasia pallida*. In addition, using a novel technique to quantify cnida discharge we demonstrate that these chemosensitizers act through at least two distinct classes of receptors. One class is broadly specific toward a variety of amino and imino acids and histamine ($K_{0.5}$ = 11–30 *nM*), but is competitively inhibited by antihistamines (K_i = 0.1–7.4 μM). A second class is specific for N-acetylated sugars ($K_{0.5}$ = 0.1–1.5 μM), but not affected by antihistamines. Presumably, these chemoreceptors detect specific substances from potential prey. Thus, cnidocytes are sensitized to discharge their cnidae in response to mechanical stimuli originating from the prey.

Introduction

Cnidocytes are secretory and sensory cells of cnidarians. They are located primarily on the tentacles of these animals. The cnidae, including the more commonly known nematocysts (Mariscal, 1984), develop within the cnidocytes and await appropriate stimuli to effect their discharge.

Cnidae are highly structured secretory products consisting of a small (5–200 μm), disulfide cross-linked (Blanquet and Lenhoff, 1966), collagen-like (Lenhoff *et al.*, 1957) capsule containing a hollow and eversible tubule continuous with the wall of the capsule (Cormier and Hessinger, 1980). They function primarily to capture prey (Ewer, 1947). The tubules of some cnidae, including certain nematocysts, evert rapidly (Holstein and Tardent, 1984) with enough force to penetrate prey and inject a lethal venom (Hessinger *et al.*, 1973; Tamkun and Hessinger, 1981). The tubules of other cnidae, including the spirocysts, are adhesive. They function to hold prey to the tentacles (Mariscal, 1984).

Cnida discharge involves the eversion of the tubule (Skaer and Picken, 1965) following proper stimulation of the cnidocyte. Parker and van Alstyne (1932) first demonstrated in the sea anenone, *Metridium senile*, and in the Portuguese Man-of-War, *Physalia physalis*, that *in situ* discharge of cnidae requires chemical stimulation and postulated the existence of chemoreceptors on cnidocytes. Subsequently, Pantin (1942) showed that discharge of *Anemonia sulcata* cnidae requires both chemical and tactile stimuli. Lubbock (1979) attempted to broadly identify the substances that sensitize cnidocytes to tactile triggering of cnida discharge by qualitatively testing 32 different high molecular weight, biological substances on the sea anemone, *Stichodactyla haddoni*.

In this report, using asexually cloned and cultured (Hessinger and Hessinger, 1981) sea anemones (*Aiptasia pallida*), we identify two groups of naturally occurring substances that chemosensitize cnidocytes for discharge. In addition, we show that the effects of these sensitizing substances are mediated by at least two distinct classes of chemoreceptor.

Materials and Methods

Maintenance of sea anemones

Sea anemones were cultured in natural seawater obtained from the Kerckoff Marine Laboratory of the Cali-

Received 4 November 1987; accepted 25 January 1988.

[1] To whom all correspondence should be addressed.

fornia Institute of Technology in Corona del Mar, California. The animals used in these experiments were asexually cloned *Aiptasia pallida*, North Carolina strain (Hessinger and Hessinger, 1981). Clonemates of similar size and age were selected and individually reared in finger bowls containing approximately 250 ml natural seawater. Anemones were daily fed to repletion on freshly hatched *Artemia* nauplii (Hessinger and Hessinger, 1981) and maintained at 24 ± 1°C under a 12/12 h photoperiod using white fluorescent lights at an intensity of 5500 lux.

Experimental animals and test solutions

A group of animals was starved for 72 h prior to each experiment. These animals were kept under constant fluorescent light at 4500 lux during the last 48 h of the starvation period. This seemed to enhance uniformity of anemone behavior and cnidocyte responsiveness in experimental situations. Immediately before experimentation the animals were gently rinsed with fresh seawater to remove soluble wastes.

All test solutions were made in filtered (Whatman type 1), natural seawater adjusted to pH 7.65 with 1 *N* HCl or NaOH. Histamine, amino acids, N-acetylneuraminic acid, N-acetylglucosamine, bovine submaxillary mucin, and most other chemicals were purchased (Sigma, St. Louis, Missouri). Diphenhydramine and cimetidine were purchased from Parke-Davis (Morris Plains, New Jersey) and Smith, Kline and French (Philadelphia, Pennsylvania), respectively.

Qualitative and quantitative assays of discharge of cnidae

Assays of cnida discharge were based on the degree of adherence of sea anemone tentacles to suitable test objects. Such adherence is mediated by the everted tubules of cnidae such as the spirocysts and the microbasic p-mastigophore nematocysts, which, respectively, attach to and penetrate test objects. The extent to which tentacles adhere to test objects has been used to qualitatively detect the discharge of cnidae (Williams, 1968; Lubbock, 1979). We developed two methods for detecting and measuring discharge of cnidae by cnidocytes in anemone tentacles: a qualitative method for screening many naturally occurring biological substances for their ability to chemosensitize tentacle cnidocytes, and a sensitive quantitative method for studying dose-response relationships of chemosensitizers.

Qualitative screening assay. We qualitatively assessed the adherence of sea anemone tentacles to clean glass rods (1 mm diam) and to gelatin (30%, w/v) and to agarose (1%, w/v) pellets (5 mm diam and 3 mm length) fastened to a thin, steel wire wand. The tip of each glass rod was immersed in the test solution for about one min, and then air-dried. Pellets were soaked for two min either in filtered seawater (negative controls) or in test solutions. Glass rods and pellets on wire wands were presented by hand to the tip of one sea anemone tentacle for five seconds and then gently withdrawn. The response of cnidocytes on the tentacles to the combined chemical and tactile stimuli was observed and graded semi-quantitatively on the basis of strength of tentacle adhesion to the test object in relation to negative controls. Adherence was qualitatively rated as 0 (none), 1 (slight to moderate), and 2 (strong). Negative controls were rated as 0. For each substance tested, we computed a weighted average score by combining scores for pellets and glass rods by first multiplying the possible scores (0, 1, and 2) by the total number of tentacles tested giving that score (a, b, and c, respectively), then adding each of those values (0a + 1b + 2c), and dividing by the total number of tentacles tested (a + b + c). The substances tested were ranked according to the ability to chemosensitize the tentacles as determined by their weighted average scores. Clean test objects were used as negative controls and both bovine submaxillary and gastric mucin were used as positive controls, giving maximum responses.

We initially screened a wide variety of substances for the ability to sensitize sea anemone cnidocytes to tactile triggering of cnida discharge. The substances included more than 60 biological or biologically active compounds, including most of the 32 compounds originally tested by Lubbock (1979), within five major categories: (i) proteins and glycoproteins; (ii) amino compounds; (iii) monosaccharides; (iv) poly- and mucopolysaccharides; and (v) lipids. Different animals were individually tested for each compound. Concentrations of the tested substances were varied depending on the ability of these substances to adhere to the glass rods. The following concentrations were used: proteins and glycoproteins (1% w/v); amino compounds (1% w/v); N-acetylated sugars (0.1 *M*) and all other monosaccharides (1 *M*), except amygdalin (1%); agarose (0.5% w/v); dextran and dextran sulphate (60% w/v); glycogen (30% w/v); sodium polypectate and starch (15% w/v); heparin (42% w/v); chondroitin-6-sulphate (30% w/v); hyaluronic acid (1.9% w/v); all lipids (0.1% w/v), except lysolecithin, sphingomyelin, and phosphatidyl ethanolamine (5 mg/ml). All lipids were dissolved in ethanol and briefly air-dried on pellets and completely air-dried on glass rods.

Quantitative assay. We developed a more accurate and sensitive measurement of tentacle adherence by using small nylon beads attached to a force-transducer (strain guage). Clean nylon beads measuring 0.80 ± 0.01 mm diameter were coated with a thin layer (0.06 mm) of gelatin (30% w/v) and stored for no more than 24 h at 4°C until used. Experiments were performed at 24°C by

exposing single anemones to 250 ml of test solution in a finger bowl. Animals were allowed to recover from the physical disruption of changing the medium for ten min before measurements were taken. Each probe was used on four separate tentacles and each anemone was used for a maximum of 20 measurements. Measurements taken in the absence of sensitizers were subtracted from measurements taken in the presence of sensitizer to give corrected values for the effect of the chemosensitizer alone. Measurements of adhesive force were made with the coated beads attached to the strain gauge (Grass model FT-03) via a narrow steel shaft. To maximize sensitivity the resistance springs were removed from the strain gauge. A sensitivity of 1 mg with 5% variation was achieved. Adhesive force measurements are expressed in hybrid units of milligram-force (mgf), rather than dynes (or newtons), since there is negligible acceleration, thereby making contributions from Newton's second law insignificant (Miller, 1959). Calibrations were obtained with weight standards and data were collected using a chart recorder. Linear regression analyses of data to determine such dose-response parameters as the maximum response (E_{max}), the concentration of agonist that produces a half-maximum response ($K_{0.5}$), and the molar disassociation constant of a competitive inhibitor (K_i), were performed using the GRAFPAC graphics computer program (Dorgan and Hessinger, 1984).

Results

Qualitative screening

We qualitatively screened more than 60 different biochemicals for the ability to chemosensitize tentacle cnidocytes to tactile triggering of discharge. Substances tested included a wide variety of proteins and glycoproteins, amino compounds, saccharides, poly- and mucopolysaccharides, and lipids (see Materials and Methods). Three types of test objects were used to present simultaneously chemical and mechanical stimuli to the anemone tentacles: gelatin pellets, agarose pellets, and glass rods. Since the results of using gelatin and agarose pellets to test various substances were quite similar, we have combined these data in Tables I through IV. The data obtained by using glass rods were somewhat different and, therefore, have been presented separately on the same tables. Weighted averages from all three types of test objects are also presented and used to rank the abilities of the substances tested to chemosensitize cnidocytes for discharge.

Proteins. The cnidocyte responses to various proteins, glycoproteins, and mucins (Table I) were either moderate (0.8–1.2) or strong (1.7–2.0). All of the tested glycoproteins and mucins produced maximum responses of 2.0, as did one non-glycosylated protein, namely poly-L-lysine. Weaker responses were elicited only by non-glycosylated proteins.

Amino compounds. Responses to amino compounds fell within three categories (Table II). No significant responses were elicited by any of the three tested antihistamines or by reduced glutathione. Strong responses (1.7) occurred in response to leucine, proline, glutamine, and histamine, while most other tested amino compounds, including glycine and alanine, elicited moderate responses (0.7–1.0).

Mono-, poly-, and mucopolysaccharides. Responses to various monosaccharides and mucopolysaccharides also varied (Table III). The tested amino sugars evoked no cnidocyte response, while glucose, galactose, and inositol produced slight to moderate responses (0.4–1.0). On the other hand, N-acetylgalactosamine and N-acetylglucosamine produced moderately strong responses, while N-acetylneuraminic acid, amygdalin and fucose produced maximum responses.

Of the tested mucopolysaccharides, anemones responded moderately to chondroitin sulphate and strongly to hyaluronic acid. Heparin, which has N-sulphates on C-2 in place of N-acetyl groups, had no activity. We were unable to test chitin, a linear polymer of N-acetylglucosamine, due to its insolubility in water. None of the tested polysaccharides including agarose, dextran sulphate, glycogen, sodium polypectate, and starch, showed any sensitizing effect (data not shown).

Lipids. None of the lipids tested evoked more than a moderate response, if at all (Table IV).

From this survey it appears that two broad groups of low molecular weight substances are identifiable that chemosensitize tentacle cnidocytes: the N-acetylated sugars and a wide variety of simple amino compounds. A variety of high molecular weight substances containing N-acetylated sugars also sensitize cnidocytes. Of these, mucins, glycoproteins and certain acidic mucopolysaccharides are the most potent.

Quantitative analysis

Dose-response parameters. To accurately quantify the relative number of cnidae that discharged in response to selected chemosensitizing substances, we measured the force (mgf) required to separate the stimulated tentacle from a probe consisting of a gelatin-coated nylon bead attached to a force-transducer. We assume this adhesive force to be directly proportional to the number of cnidae that discharged and adhered to the coated nylon bead.

Dose-response curves of all tested sensitizers were biphasic, showing a sigmoidal region of sensitization at lower concentrations of sensitizer, a maximum response at higher concentrations, and a downward response at still higher concentrations. The dose-response curves for

Table I

Responses of cnidocytes of Aiptasia pallida *tentacles to various proteins, glycoproteins, and mucins*

Compound	Pellet			Glass rod			Weighted averages
	0	1	2	0	1	2	
None	19	1	—	10	—	—	0.03
α-casein	6	14	—	—	10	—	0.80
Cytochrome C (horse)	2	18	—	—	10	—	0.93
Pepsin (porcine)	—	20	—	—	10	—	1.00
Trypsin	—	20	—	—	10	—	1.00
Haemoglobin	4	16	—	—	—	10	1.20
Lysozyme (egg white)	—	—	20	—	10	—	1.67
Myoglobin (equine)	4	—	16	—	—	10	1.73
Ovalbumin (hen)*	—	—	20	—	—	10	2.00
Polylysine	—	—	20	—	—	10	2.00
α-globulin (bovine)*	—	—	20	—	—	10	2.00
Serum albumin*	—	—	20	—	—	10	2.00
Submaxillary mucin*	—	—	20	—	—	10	2.00
Gastric mucin*	—	—	20	—	—	10	2.00

Responses are graded as 0 (none), 1 (slight), or 2 (strong). A total of 30 tentacles are tested for each compound: ten times each on agarose pellets, gelatin pellets, and glass rods. Results obtained with agarose and gelatin pellets are combined. Weighted averages represent combined scores for all 30 trials (see Materials and Methods). * Indicates glycoproteins.

glycine and N-acetylneuraminic acid (NANA) are typical of two general types of chemosensitizer (Fig. 1), each showing a distinct maximum response or effect (E_{max}) as well as a concentration in the sensitization region producing a half-maximal response ($K_{0.5}$), but with the NANA response showing a distinctively narrow and reproducible peak. Similarly shaped curves were obtained for alanine, glutamine, proline, and histamine, on the one hand, and for N-acetylglucosamine, and bovine submaxillary mucin, on the other.

Data from the sensitization region of these dose-response curves can be linearized on double-reciprocal

Table II

Responses of cnidocytes of Aiptasia pallida *tentacles to various non-protein amino compounds*

Compound	Pellet			Glass rod			Weighted averages
	0	1	2	0	1	2	
Diphenhydramine	20	—	—	10	—	—	0
Tripelennamine	20	—	—	10	—	—	0
Cimetidine	20	—	—	10	—	—	0
Glutathione (reduced)	14	6	—	10	—	—	0.20
Aspartic acid	—	20	—	10	—	—	0.67
Glutamic acid	—	20	—	10	—	—	0.67
Valine	10	10	—	—	10	—	0.67
Lysine	14	—	6	—	10	—	0.73
Serine	6	14	—	—	10	—	0.80
Alanine	2	18	—	—	10	—	0.93
Glycine	—	20	—	—	10	—	1.00
Cysteine	—	20	—	—	10	—	1.00
Histidine	—	20	—	—	10	—	1.00
Hydroxyproline	—	20	—	—	10	—	1.00
Hydroxylysine	—	20	—	—	10	—	1.00
Leucine	—	—	20	—	10	—	1.67
Proline	—	—	20	—	10	—	1.67
Glutamine	—	—	20	—	10	—	1.67
Histamine	—	—	20	—	10	—	1.67

Table III

Responses of cnidocytes of Aiptasia pallida *tentacles to monosaccharides and to mucopolysaccharides*

Compound	Pellet			Glass rod			Weighted averages
	0	1	2	0	1	2	
A. Monosaccharides:							
Galactosamine	20	—	—	10	—	—	0
Glucosamine	20	—	—	10	—	—	0
Galactose	18	2	—	—	10	—	0.40
Glucose	8	12	—	7	3	—	0.50
Inositol	—	20	—	—	10	—	1.00
N-Acetylgalactosamine	4	—	16	3	—	7	1.53
N-Acetylglucosamine	2	—	18	—	—	10	1.87
N-Acetylneuraminic acid	—	—	20	—	—	10	2.00
Amygdalin	—	—	20	—	—	10	2.00
Fucose	—	—	20	—	—	10	2.00
B. Mucopolysaccharides:							
Heparin	20	—	—	10	—	—	0
Chondroitin-6-sulphate	—	20	—	—	10	—	1.00
Hyaluronic acid	—	—	20	—	—	10	2.00

plots using linear regression analyses to calculate $K_{0.5}$ and E_{max} values (Table V). The $K_{0.5}$ values from these curves fall into two numerical groups: values of 1–3 $\times 10^{-2}$ μM for the amino sensitizers; and values one to two orders of magnitude higher (0.1–1.5 μM) for the N-acetylated sugars and mucin. These differences in dose-responsiveness, along with the obvious chemical differences between these two groups of sensitizers, suggest that these two groups of sensitizers occupy different receptors.

Is there more than one type of cnidocyte chemoreceptor? To determine whether more than one type of cnidocyte chemoreceptor exists, we tested analogues of various sensitizers that might block the cnidocyte response to one type of sensitizer but not the other. Our procedure involved testing such substances for the ability to block discharge of cnidae in the presence of a known sensitizing agent (agonist), while not themselves sensitizing the cnidocytes. We found that certain antihistamines (diphenhydramine, tripelennamine, and cimetidine) fit these criteria, blocking sensitization by amino agonists, but not sensitization to N-acetylated sugars (Table V). In particular, low concentrations of diphenhydramine displaced the sigmoidal region of the dose-response curve to the right for each of the amino agonists. Thus, increasing levels of diphenhydramine progressively shifted the $K_{0.5}$ to higher agonist concentrations on dose-response curves and on double-reciprocal plots (Fig. 2), while not affecting E_{max} values. This inhibitory effect is reversible and characteristic of receptor systems blocked by competitive inhibitors and antagonists (Segel, 1976; Goldstein *et al.*, 1974).

Table IV

Responses of cnidocytes of Aiptasia pallida *tentacles to various lipids*

Compound	Pellet			Glass rod			Weighted averages
	0	1	2	0	1	2	
Phosphatidyl ethanolamine*	20	—	—	10	—	—	0
Squalene	16	4	—	10	—	—	0.13
Sphingomyelin	16	4	—	10	—	—	0.13
Lysophosphatidyl choline	14	6	—	—	10	—	0.53
Gangliosides (brain)	6	14	—	—	10	—	0.80
Phosphatidyl choline (egg yolk)	—	20	—	—	10	—	1.00
Dipalmitoyl phosphatidyl choline	6	8	6	—	10	—	1.00

* Also inactive were: cholesterol, cholesterol palmitate, testosterone, palmitic acid, oleic acid, and egg yolk lysolecithin.

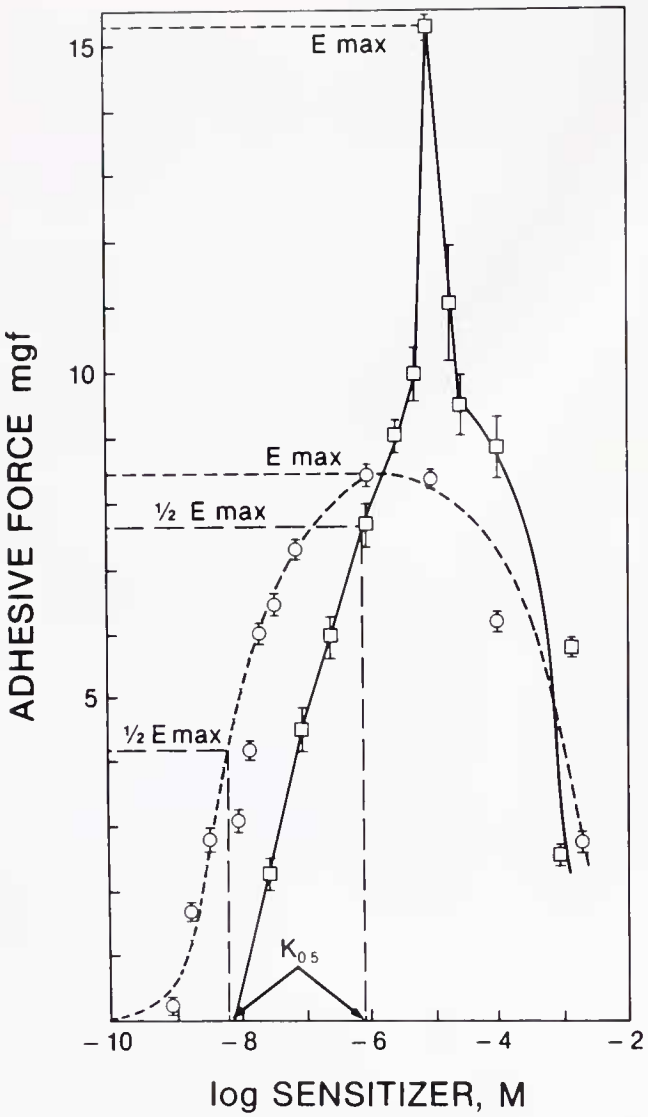

Figure 1. Dose-response curves of the cnidocyte response to glycine and N-acetylneuraminic acid (NANA). The dose-response curves for glycine (dashed line; mean n = 49, ranging from 31 to 72) and NANA (solid line; mean n = 90, ranging from 53 to 150) are typical of two types of chemosensitizing agents. Results are expressed as means of adhesive force (mgf) after correcting for adhesion with seawater alone, with vertical bars representing standard errors of means (95% confidence limits).

Dixon-type plots (Dixon, 1953) of the reciprocal of adhesive force *versus* inhibitor concentration yield straight lines for different concentrations of amino agonists (Fig. 3). These lines intersect at a common point that is indicative of competitive inhibition (Segel, 1976) and which gives the K_i for the antagonist.

Discussion

Results of our qualitative survey of more than 60 biochemicals for the ability to chemosensitize *A. pallida* cnidocytes confirm and extend the observations of Lubbock (1979) who used the large anemone *Stichodactyla haddoni*. Lubbock, using glass rods to present simultaneously the tactile stimulus and the chemical stimulus, showed that mucin and a few proteins—among 32 tested substances—allowed strong responses, while polysaccharides and lipids were virtually inactive. In the present survey we found that mucins, and specifically glycoproteins and a mucopolysaccharide, chemosensitize cnidocytes. In addition, however, we found that a wide variety of amino compounds and certain sugars strongly sensitize cnidocytes in the tentacles of *A. pallida*. Furthermore, *A. pallida* responded much more strongly to hyaluronic acid and polylysine and much less to α-casein than did *S. haddoni*.

The present study demonstrates that cnidocyte chemosensitization occurs either with the sensitizers free in solution (Table V) or adsorbed to a biologically inert gel (Tables I–IV). Furthermore, we have identified two groups of naturally occurring, low molecular weight substances that sensitize cnidocytes in the tentacles of the sea anemone, *A. pallida:* a variety of amino compounds (Table II) and three N-acetylated sugars plus amygdalin and fucose (Table III). The three N-acetylated sugars, all of which have an N-acetyl group on a hexose ring, are common constituents of glycoproteins, mucins, and mucopolysaccharides. Amygdalin, on the other hand, is a glycoside having a malenonitrile group attached to the C-1 of a hexose. Under certain conditions (*e.g.*, acid hydrolysis or alkaline peroxide attack) nitriles are converted to amides. In the case of amygdalin, the amide would be placed very close to C-2 of the hexose forming a close structural analogue to the N-acetyl hexoses. Fucose, while structurally dissimilar to N-acetylated sugars, is also a common constituent of mucins and glycoproteins.

While another group of substances, the antihistamines, do not sensitize cnidocytes (Table II), they do exert effects on the tentacle cnidocytes that are characteristic of receptor systems blocked by competitive inhibitors and antagonists (Segal, 1976; Goldstein *et al.*, 1974). They (i) displace dose-response curves of the amino agonists to the right, (ii) increase the $K_{0.5}$ values of amino agonists on double-reciprocal plots while not affecting E_{max} values (Fig. 2), and (iii) produce Dixon plots that yield straight lines having a common point of intersection (Fig. 3), which gives the K_i for the antagonist (Table V) and represents the dissociation constant of the receptor-inhibitor complex (Segel, 1976; Dixon, 1953). Therefore, we conclude that the antihistamine, diphenhydramine, acts as an antagonist at the amino receptor.

Due to the similarity of K_i values determined for diphenhydramine in the presence of proline, glutamine, histamine and, possibly, alanine (Table V), it seems

Table V

Dose-response parameters of chemoreceptor-mediated cnida discharge in Aiptasia pallida *tentacles*

	E_{max} (mgf)	$K_{0.5}$ (μM)	K_i (μM)
Glycine	7.95 ± 0.53	$1.09 \times 10^{-2} \pm 0.08$	0.129 ± 0.049
Alanine	8.51 ± 0.71	$3.00 \times 10^{-2} \pm 0.31$	7.35 ± 2.23
Proline	8.62 ± 0.61	$2.74 \times 10^{-2} \pm 0.22$	3.06 ± 0.58
Glutamine	10.07 ± 0.89	$2.40 \times 10^{-2} \pm 0.21$	3.06 ± 0.45
Histamine	14.22 ± 0.44	$2.04 \times 10^{-2} \pm 0.07$	2.42 ± 0.75
N-Acetylglucosamine	9.75 ± 0.48	0.115 ± 0.008	no effect
N-Acetylneuraminic acid	14.36 ± 1.51	1.46 ± 0.25	no effect
Submaxillary mucin	13.83 ± 1.29	1.55 ± 0.18	no effect

E_{max} is the adhesive force (mgf) required to separate an adhering tentacle from the probe tip at maximum sensitization. The $K_{0.5}$ represents the molar concentration of substance that half-maximally sensitizes the tentacle cnidocytes. Values of E_{max} and $K_{0.5}$ are determined from least-square double-reciprocal plots of the sensitization region of individual dose-response curves. K_i represents the molar dissociation constant of the receptor-inhibitor complex for a competitive inhibitor (*i.e.*, diphenhydramine) and is obtained as the x-intercept from linear plots of the apparent $K_{0.5}$ as a function of inhibitor concentration (Segal, 1976). The values of all parameters are expressed as means ± standard deviations.

likely that these amino agonists occupy the same or similar chemoreceptors, whereas the K_i value for glycine is significantly different. The antihistamines, however, have no effect on the cnidocyte response to the N-acetylated sugars or to bovine submaxillary mucin (Table V).

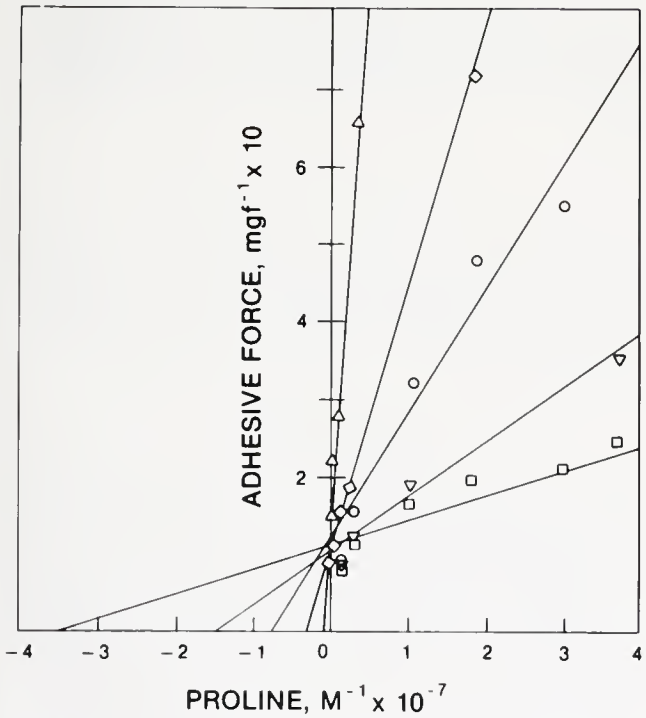

Figure 2. Double-reciprocal plots of proline-mediated sensitization in the presence of four different concentrations of diphenhydramine. The double-reciprocal plot of the sensitizing half of the biphasic dose-response curve is linear and yields $K_{0.5}$ and E_{max} values from x- and y-intercepts, respectively. Increasing concentrations of diphenhydramine cause an increase in the apparent $K_{0.5}$ value while not changing the value of E_{max}. Molar concentrations of diphenhydramine used were: no diphenhydramine (□); 5×10^{-7} (▽); 5×10^{-6} (○); 1.65×10^{-5} (◇); and 3.30×10^{-5} (△). Each data point is the mean of 60 or more tentacles with the standard errors being less than 0.002 mgf^{-1}.

We conclude that the cnidocyte sensitizing effect is mediated by surface chemoreceptors since the dose-response is saturable, and since the effect is reversible, specific, and can be competitively inhibited. We also conclude that there are at least two classes of cnidocyte chemoreceptors: (i) one class, with general specificity for a variety of amino and imino acids and histamine (Table II), but competitively inhibited by antihistamines (Figs. 2, 3); and (ii) a second class, specific for N-acetylated sugars and for high molecular weight substances bearing terminal N-acetylated sugars (Tables I, III), such as mucin, glycoproteins, and certain mucopolysaccharides, but not affected by antihistamines.

The fact that the oligosaccharide branches of bovine submaxillary mucin terminate in NANA residues (Herp *et al.*, 1979) suggests that mucin binds to the same chemoreceptor as free NANA. Furthermore, the nearly identical $K_{0.5}$ values for mucin and free NANA (Table V) suggest that a monovalent interaction of mucin with the "sugar" receptor is sufficient to chemosensitize, as opposed to requiring multivalent interaction and/or a clustering of NANA receptors.

These findings are the first to demonstrate and identify chemoreceptors involved in controlling cnidae discharge. Apparent desensitization of the receptor-mediated response of the cnidocytes is observed at high concentrations of both amino and N-acetylated sugar sensitizers. We speculate that such desensitization or sensory adaptation is a consequence either of receptor modification and/or of receptor-mediated endocytosis (Watson and Hessinger, 1987).

We propose a role for these cnidocyte chemoreceptors in the feeding process of cnidarians. Feeding by cnidarians involves two coordinated behaviors, namely, prey

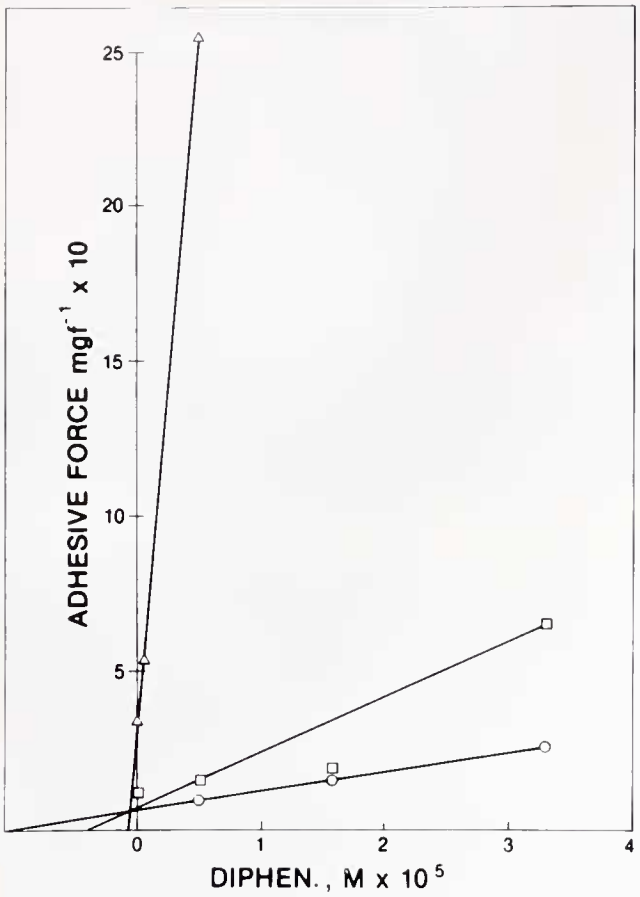

Figure 3. Dixon-type plots of proline-mediated sensitization in the presence of three different concentrations of proline. Molar concentrations of proline used were: (△) 2.7×10^{-8}; (□) 2.7×10^{-7}; and (○) 1×10^{-6}. Each data point is the mean of between 23 and 74 (average n = 40) tentacles.

capture and the feeding response. (i) Prey capture is effected by cnida discharge triggered by combined chemical and mechanical stimuli originating from the prey. Surface mucins on some prey (Daniel, 1981; Downing *et al.*, 1981) and the chitin exoskeletons of others, both of which either contain (Herp *et al.*, 1979) or are composed of (Austin *et al.*, 1981) N-acetylated sugars, may bind to one class of chemoreceptor and sensitize cnidocytes by lowering the threshold for discharging to mechanical stimulation. Upon discharge of the cnidae, the penetrant nematocysts puncture prey and inject nematocyst toxins that initially stimulate prey motor activity of the prey (Hessinger *et al.*, 1973), thereby increasing mechanical stimulation of cnidocytes. In addition, the puncture wounds caused by the penetrant nematocysts allow soluble substances, such as amino acids, to leak from the prey into the ambient medium. Some of those amino acids from the prey may sensitize additional cnidocytes via a second class of cnidocyte receptor causing additional cnidae to discharge. (ii) Following capture of the prey, the feeding response, involving a concerted movement of the tentacles to the mouth and the opening of the mouth, is also triggered by soluble substances leaking from punctured prey (Lenhoff and Heagy, 1977). In hydra the feeding response is induced by reduced glutathione (Loomis, 1955; Lenhoff, 1968; Cobb *et al.*, 1982), while in some sea anemones (Lenhoff and Heagy, 1977), including *A. pallida* (unpub. obs.), it is induced by some of the same amino compounds that sensitize cnidae to discharge. Thus, in many cnidarians, as in *A. pallida*, both prey capture and the feeding response are likely to be mediated by surface chemoreceptors that detect specific chemicals contained on and within suitable prey.

The present findings show that the control of cnida discharge can be studied at the molecular level. This cnida-cnidocyte system will be useful in unraveling both the mechanisms of sensory transduction in cnidocytes and the means by which chemical and mechanical stimuli are integrated to effect cnida discharge. The present approach to identifying and characterizing cnidocyte chemoreceptors involved in prey capture may also be useful in elucidating the mechanisms that control cnidocyte behavior as it relates to such phenomena as clonal aggression (*e.g.*, Francis, 1973) and tolerance of symbiotic anemonefish by certain sea anemones (*e.g.*, Schlichter, 1976).

Acknowledgments

We thank Drs. R. Mariscal, B. Taylor, and G. Watson for helpful discussions and comments on the manuscript. Supported in part by funds to D.A.H. from BRSG grant RR 05352-24 and NSF grant DCB-8609859.

Literature Cited

Austin, P. R., C. J. Brian, J. E. Castle, and J. P. Zikakis. 1981. Chitin: new facets of research. *Science* **212:** 749–753.

Blanquet, R., and H. M. Lenhoff. 1966. A disulphide-linked collagenous protein of nematocyst capsules. *Science* **154:** 152–153.

Cobb, M. H., W. Heagy, J. Danner, H. M. Lenhoff, and G. R. Marshall. 1982. Structural and conformational properties of peptides interacting with the glutathione receptor of hydra. *Mol. Pharmacol.* **21:** 629–636.

Cormier, S. M., and D. A. Hessinger. 1980. Cellular basis for tentacle adherence in the Portuguese Man-of-War (*Physalia physalis*). *Tissue Cell* **12:** 713–721.

Daniel, T. L. 1981. Fish mucus: *in situ* measurement of polymer drag reduction. *Biol. Bull.* **160:** 376–382.

Dorgan, L., and D. A. Hessinger. 1984. *GRAFPAC, a Graphics and Format Package for the Apple II+ (IIe) Computer.* Copyright 1984.

Downing, S. W., W. L. Salo, R. H. Spitzer, and E. A. Koch. 1981. The hagfish slime gland: a model system for studying the biology of mucus. *Science* **214:** 1143–1145.

Dixon, M. 1953. The effect of pH on the affinities of enzymes for substrates and inhibitors. *Biochem. J.* **55:** 161–170.

Ewer, R. F. 1947. On the functions and mode of action of the nematocysts of hydra. *Proc. Zool. Soc. Lond.* **117:** 365–376.

Francis, L. 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related anemones. *Biol. Bull.* **144:** 73–92.

Goldstein, A., L. Arnow, and S. M. Kalman. 1974. Pp. 85–92 in *Principles of Drug Action,* 2nd Ed. Wiley, New York.

Herp, A., A. M. Wu, and J. Moschera. 1979. Current concepts of the structure and nature of mammalian salivary mucous glycoproteins. *Mol. Cell. Biochem.* **23:** 27–44.

Hessinger, D. A., and J. A. Hessinger. 1981. Methods for rearing sea anemones in the laboratory. Pp. 153–179 in *Marine Invertebrates,* Committee on Marine Invertebrates, ed. National Academy Press, Washington, DC.

Hessinger, D. A., H. M. Lenhoff, and L. B. Kahan. 1973. Haemolytic, phospholipase A and nerve-affecting activities of sea anemone nematocyst venom. *Nature New Biol.* **241:** 125–127.

Holstein, T., and P. Tardent. 1984. An ultrahigh-speed analysis of exocytosis: nematocyst discharge. *Science* **223:** 830–832.

Lenhoff, H. M., Kline, E., and R. Hurley. 1957. A hydroxyproline-rich, intracellular collagen-like protein of *Hydra* nematocysts. *Biochim. Biophys. Acta* **26:** 204–205.

Lenhoff, H. M. 1968. Behavior, hormones and hydra. *Science* **161:** 434–442.

Lenhoff, H. M., and W. Heagy. 1977. Aquatic invertebrate model systems for study of receptor activation and evolution of receptor proteins. *Ann. Rev. Pharmacol. Toxicol.* **17:** 243–258.

Loomis, W. F. 1955. Glutathione control of the specific feeding reactions of hydra. *Ann. N.Y. Acad. Sci.* **62:** 209–228.

Lubbock, R. 1979. Chemical recognition and nematocyte excitation in a sea anemone. *J. Exp. Biol.* **83:** 283–292.

Mariscal, R. N. 1984. Cnidaria: Cnidae. Pp. 57–68 in *Biology of the Integement,* Vol. I. Invertebrates. Springer-Verlag, Berlin.

Miller, F., Jr. 1959. Pp. 58 in *College Physics,* Harcourt, Brace and World, New York.

Pantin, C. F. A. 1942. The excitation of nematocysts. *J. Exp. Biol.* **19:** 294–310.

Parker, G. H., and M. A. Van Alstyne. 1932. The control and discharge of nematocysts, especially in *Metridium* and *Physalia. J. Exp. Zool.* **63:** 329–344.

Schlichter, D. 1976. Macromolecular mimicry: substances released by sea anemones and their role in the protection of anemone fishes. Pp. 433–441 in *Coelenterate Ecology and Behavior,* G. O. Mackie, ed. Plenum, New York.

Segel, I. H. 1976. Pp. 246–252 in *Biochemical Calculations,* 2nd ed. Wiley, New York.

Skaer, R. J., and L. E. R. Picken. 1965. The structure of the nematocyst thread and the geometry of discharge in *Cornyactis viridis* (Allman). *Philos. Trans. R. Soc. Lond.* **250:** 131–164.

Tamkun, M. M., and D. A. Hessinger. 1981. Isolation and partial characterization of a hemolytic and toxic protein from the nematocyst venom of the Portuguese Man-of-War, *Physalia physalis. Biochim. Biophys. Acta* **667:** 87–98.

Watson, G., and D. A. Hessinger. 1987. Receptor-mediated endocytosis of a purported chemoreceptor involved in triggering the discharge of cnidae in a sea anemone tentacle. *Tissue Cell* **19:** 747–755.

Williams, R. B. 1968. Control of the discharge of cnidae in *Diadumene luciae* (Verill). *Nature* **219:** 959–960.